

EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNG
GTSHNQRFMDFRFTISVDKSTSTAYMQMNSLRAEDTAVYCARWRGLNYGFDVRYFD
VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

FIG. 1A

DIQMTQSPSSLSASVGDRTTTCRASQDINNVLNWNYYQQKPGKAPKLLINYTSTLHSGVP
SRFSGSGGTDYTLTISSLPEDFATYYCQQGNTLPFTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST
LTLSKADYEEKHKVYACEVTHQGLSPVTKSFNRGEC

FIG. 1B

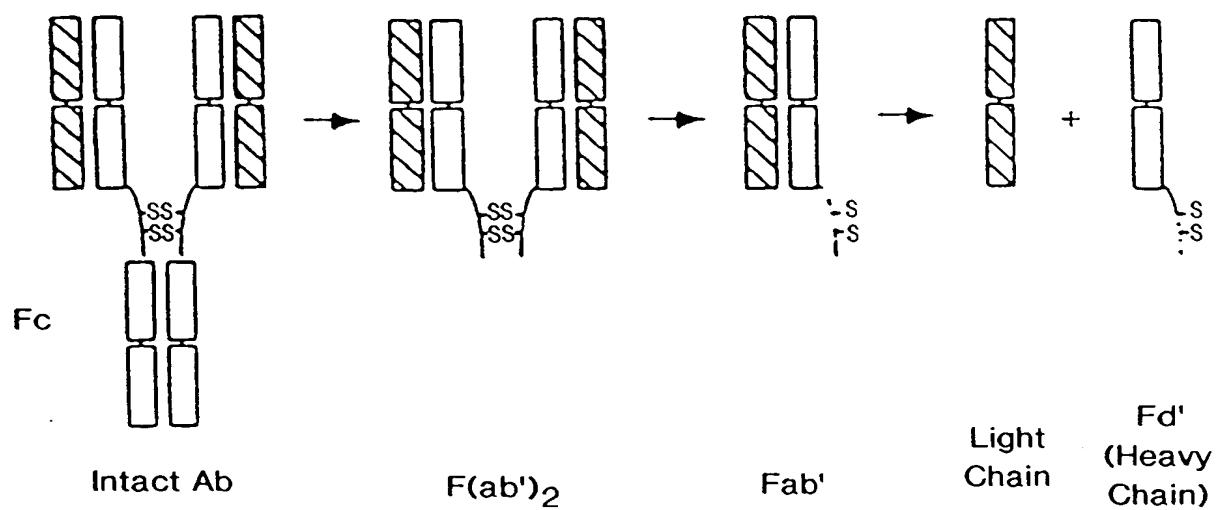


FIG. 2A

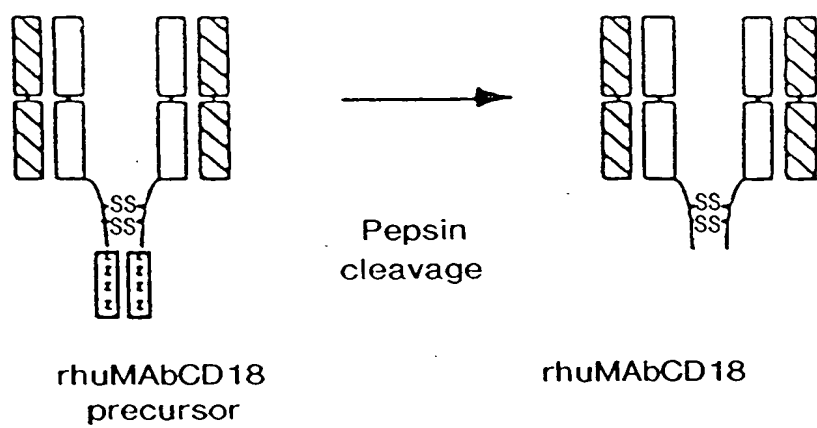


FIG. 2B

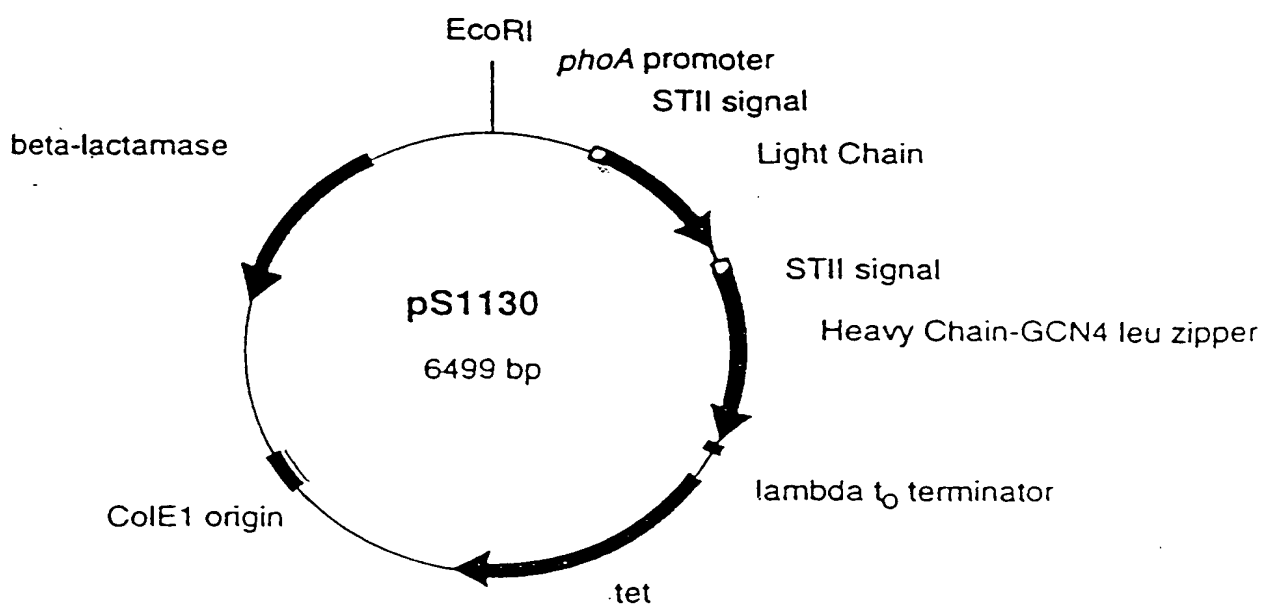


FIG. 3

1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA
 61 GTTGTATTAT AAGCTTTGGA GATTATCGTC ACTGCAATGC TTCGCAATAT GGCGCAAAAT
 121 GACCAACAGC GGTGATTGA TCAGGTAGAG GGGGCGCTGT ACGAGGTAAA GCCCGATGCC
 181 AGCATTCCTG ACGACGATAC GGAGCTGCTG CGCGATTACG TAAAGAAGTT ATTGAAGCAT
 241 CCTCGTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGTCATA AAGTTGTCAC GGCCGAGACT
 301 TATAGTCGCT TTGTTTTTAT TTTTAAATGT ATTTGTAAC TGAATTCGAG CTCGCCGGGG
 361 ATCCTCTAGA GGTGAGGTG ATTTT ATG AAA AAG AAT ATC GCA TTT CTT CTT
 -23 M K K N I A F L L
 413 GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAC GCG TAC GCT GAT ATC
 -14 A S M F V F S I A T N A Y A D I
 461 CAG ATG ACC CAG TCC CCG AGC TCC CTG TCC GCC TCT GTG GGC GAT AGG
 3 Q M T Q S P S S L S A S V G D R
 509 GTC ACC ATC ACC TGT CGT GCC AGT CAG GAC ATC AAC AAT TAT CTG AAC
 19 V T I T C R A S Q D I N N Y L N
 557 TGG TAT CAA CAG AAA CCA GGA AAA GCT CCG AAA CTA CTG ATT TAC TAT
 35 W Y Q Q K P G K A P K L L I Y Y
 605 ACC TCC ACC CTC CAC TCT GGA GTC CCT TCT CGC TTC TCT GGT TCT GGT
 51 T S T L H S G V P S R F S G S G
 653 TCT GGG ACG GAT TAC ACT CTG ACC ATC AGC AGT CTG CAA CCG GAG GAC
 67 S G T D Y T L T I S S L Q P E D
 701 TTC GCA ACT TAT TAC TGT CAG CAA GGT AAT ACT CTG CCG CCG ACG TTC
 83 F A T Y Y C Q Q G N T L P P T F
 749 GGA CAG GGC ACG AAG GTG GAG ATC AAA CGA ACT GTG GCT GCA CCA TCT
 99 G Q G T K V E I K R T V A A P S
 797 GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC
 115 V F I F P P S D E Q L K S G T A
 845 TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA
 131 S V V C L L N N F Y P R E A K V
 893 CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
 147 Q W K V D N A L Q S G N S Q E S
 941 GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC
 163 V T E Q D S K D S T Y S L S S T
 989 CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC
 179 L T L S K A D Y E K H K V Y A C
 1037 GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC
 195 E V T H Q G L S S P V T K S F N
 1085 AGG GGA GAG TGT TAA G CTGATCCTCT ACGCCGGACG CATCGTGGCG
 211 R G E C

FIG. 4A

1131 CTAGTACGCA AGTTCACGTA AAAACGGTAT CTAGAGGTTG AGGTGATTTT ATG AAA
 -23 M K

1187 AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT
 -21 K N I A F L L A S M F V F S I A

1235 ACA AAC GCG TAC GCT GAG GTT CAG CTG GTG GAG TCT GGC GGT GGC CTG
 -5 T N A Y A E V Q L V E S G G G L

1283 GTG CAG CCA GGG GGC TCA CTC CGT TTG TCC TGT GCA ACT TCT GGC TAC
 12 V Q P G G S L R L S C A T S G Y

1331 ACC TTT ACC GAA TAC ACT ATG CAC TGG ATG CGT CAG GCC CCG GGT AAG
 28 T F T E Y T M H W M R Q A P G K

1379 GGC CTG GAA TGG GTT GCA GGG ATT AAT CCT AAA AAC GGT GGT ACC AGC
 44 G L E W V A G I N P K N G G T S

1427 CAC AAC CAG AGG TTC ATG GAC CGT TTC ACT ATA AGC GTA GAT AAA TCC
 60 H N Q R F M D R F T I S V D K S

1475 ACC AGT ACA GCC TAC ATG CAA ATG AAC AGC CTG CGT GCT GAG GAC ACT
 76 T S T A Y M Q M N S L R A E D T

1523 GCC GTC TAT TAT TGT GCT AGA TGG CGA GGC CTG AAC TAC GGC TTT GAC
 92 A V Y Y C A R W R G L N Y G F D

1571 GTC CGT TAT TTT GAC GTC TGG GGT CAA GGA ACC CTG GTC ACC GTC TCC
 108 V R Y F D V W G Q G T L V T V S

1619 TCG GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC
 124 S A S T K G P S V F P L A P S S

1667 AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC
 140 K S T S G G T A A L G C L V K D

1715 TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC
 156 Y F P E P V T V S W N S G A L T

1763 AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC
 172 S G V H T F P A V L Q S S G L Y

1811 TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
 188 S L S S V V T V P S S S L G T Q

1859 ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTC GAC
 204 T Y I C N V N H K P S N T K V D

1907 AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCG CCG
 220 K K V E P K S C D K T H T C P P

1955 TGC CCA GCA CCA GAA CTG CTG GGC GGC CGC ATG AAA CAG CTA GAG GAC
 236 C P A P E L L G G R M K Q L E D

2003 AAG GTC GAA GAG CTA CTC TCC AAG AAC TAC CAC CTA GAG AAT GAA GTG
 252 K V E E L L S K N Y H L E N E V

2051 GCA AGA CTC AAA AAG CTT GTC GGG GAG CGC TAA GCATGCG ACGGCCCTAG
 268 A R L K K L V G E R

2101 AGTCCCTAAC GCTCGGTTGC CGCCGGGCGT TTTTATTGT TAA

FIG. 4B

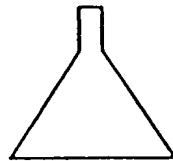
<u>Strain</u>	<u>Genotype</u>
W3110	K-12 F ⁻ lambda ⁻ IN _{rmD-rmE1}
↓	
1A2	W3110 Δ <i>fhuA</i>
↓	
7C1	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>)
↓	
16C9	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>) <i>deoC</i>
↓	
23E3	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>) <i>deoC</i> Δ <i>degP</i>
↓	
33B6	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>) <i>deoC</i> Δ <i>degP</i> <i>ilvG</i>
↓	
49B2	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>) <i>deoC</i> Δ <i>degP</i> <i>ilvG</i> Δ <i>fucP</i>
↓	
49A5	W3110 Δ <i>fhuA</i> Δ <i>phoA</i> Δ(<i>argF-lac</i>) <i>deoC</i> Δ <i>degP</i> <i>ilvG</i> Δ <i>fucP</i> Δ <i>malE</i>

FIG. 5

VIAL FROM
WORKING CELL BANK
OR
MASTER CELL BANK



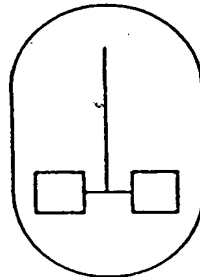
PRIMARY INOCULUM
SHAKE FLASK
MEDIUM



14–18 hours
temperature controlled



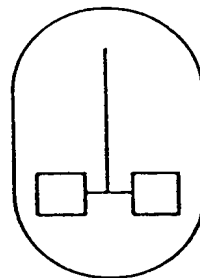
SECONDARY INOCULUM,
SECONDARY MEDIUM



7–17 hours
temperature and pH controlled
transferred at 10–25 OD₅₅₀



PRODUCTION VESSEL,
PRODUCTION MEDIUM



temperature and pH controlled
controlled nutrient feeds
harvested at 60–84 hours



HARVEST BY CENTRIFUGATION



FREEZING OF CELLS

FIG. 6

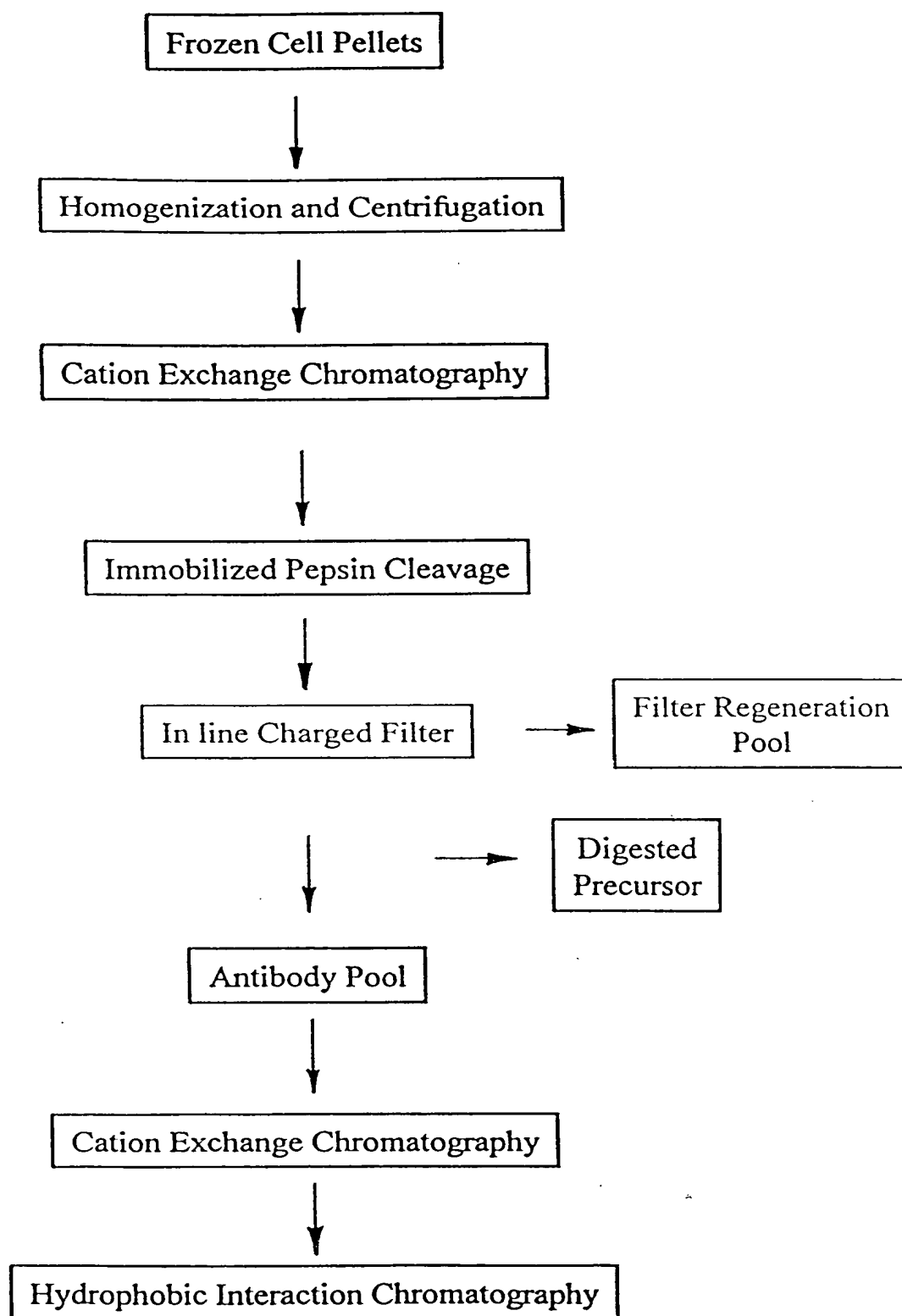


FIG. 7